ATENT COOPERATION TR Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 November 2000 (23.11.00)	
International application No. PCT/FI00/00375	**Applicant's or agent's file reference** 31863
International filing date (day/month/year) 28 April 2000 (28.04.00)	**Priority date** (day/month/year) 30 April 1999 (30.04.99)
Applicant SIPPONEN, Pentti et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

25 October 2000 (25.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer R. E. Stoffel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

FI0000375

ATENT COOPERATION TR Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-00120 Helsinki
FINLANDE

Date of mailing (day/month/year)
08 March 2001 (08.03.01)

Applicant's or agent's file reference
31863

IMPORTANT NOTIFICATION

International application No.
PCT/FI00/00375

International filing date (day/month/year)
28 April 2000 (28.04.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
LOCUS GENEX OY
Laippatie 1
FIN-00880 Helsinki
Finland

State of Nationality: FI
State of Residence: FI
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
BIOHIT OYJ
Laippatie 1
FIN-00880 Helsinki
Finland

State of Nationality: FI
State of Residence: FI
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
F. Baechler
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

09/936865

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 31863	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FI00/00375	International filing date *(day/month/year)* 28.04.2000	Priority date *(day/month/year)* 30.04.2000
International Patent Classification (IPC) or national classification and IPC7 C 12 Q 1/34, C 12 Q 1/54		
Applicant BIOHIT OYJ et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.10.2000	25.07.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88 Telex 17978 PATOREG-S	Authorized officer Hampus Rystedt/BS Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI00/00375

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7	YES
	Claims	8-11	NO
Inventive step (IS)	Claims	1-7	YES
	Claims	8-11	NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to a method for direct determination of disaccharidase activity in a duodenum biopsy sample using a test kit. The test kit comprises a disaccharide and a glucose/galactose assay system.

The following documents are considered relevant:
D1: Smith, J.A. et al, Small bowel biopsy for disaccharidase levels: evidence that endoscopic forceps biopsy can replace the Crosby capsule, Clinica Chimica Acta, 1989, vol 183, pp 317-322.
D2: Iqbal, T.H. et al, Small intestinal lactase status, frequency distribution of enzyme activity and milk intake in a multi-ethnic population, Clinical Nutrition, 1996, vol 15, pp 297-302
D3: Dahlqvist, A., Method for assay of intestinal disaccharidases, Anal Biochem, 1964, vol 7, pp 18-25.
D4: EP-A1-72450

D1 and D2 disclose methods for estimating lactase, sucrase and maltase activities in biopsy samples from the duodenum of patients with possible disaccharide intolerance. The methods use an assay system comprising disaccharides, glucose oxidase and a colour-forming agent. Both D1 and D2 refer to the method used as being a variant of the method originally described i D3.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D1-D3 differs from the present application in the homogenization step of the biopsy samples. In D1 and D3 it is expressly stated that the biopsy samples are homogenized; D2 refers to "an automated modification of Dahlqvist's method", it is not mentioned in D2 that it could be possible to omit the homogenization step. Claim 1 of the present application characterizes the method by a step comprising "contacting the said biopsy sample *as such* with a substrate medium...", i.e. the biopsy sample should not be homogenized. It is not considered obvious to a person skilled in the art that the samples need not be homogenized. Claims 1-7 are therefore considered inventive.

However, this characterizing feature is not present in the claims relating to a kit for carrying out the method, i.e. claims 8-11. Such kits are known through e.g. D4. Claims 8-11 consequently lack novelty with regard to D4.

A-W
09/936865

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 31863

Box No. I TITLE OF INVENTION

Method for the determination of disaccharidases and kit therefor

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LOCUS GENEX OY
Laippatie 1
FIN-00880 Helsinki
Finland

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: Finland

State *(that is, country)* of residence: Finland

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SIPPONEN, Pentti
Käärmesaarentie 4 A
FIN-02160 Espoo
Finland

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Finland

State *(that is, country)* of residence: Finland

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-00120 Helsinki
Finland

Telephone No. +358 9 612 6120

Facsimile No. +358 9 640 575

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SUOVANIEMI, Osmo
Kulopolku 6
FIN-00570 Helsinki
Finland

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Finland

State *(that is, country)* of residence: Finland

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

TAMMINEN, Jani
Ormusmäentie 8 C 45
FIN-00700 Helsinki
Finland

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Finland

State *(that is, country)* of residence: Finland

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

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☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
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☒ CN China
☒ CR Costa Rica
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☒ US United States of America
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☒ VN Viet Nam
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Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ DZ Algeria
☒ AG Antigua and Barbuda

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

See Notes to the request form

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (30.04.1999) 30 April 1999	990990	Finland		
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/SE

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	7
claims	:	2
abstract	:	1
drawings	:	
sequence listing part of description	:	
Total number of sheets	:	14

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Copy of FI Office Action of 14.9.1999

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

OY JALO ANT-WUORINEN AB

Eva Grew

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/34, 1/54

A1

(11) International Publication Number: WO 00/66765

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/FI00/00375

(22) International Filing Date: 28 April 2000 (28.04.00)

(30) Priority Data:
990990 30 April 1999 (30.04.99) FI

(71) Applicant *(for all designated States except US)*: LOCUS GENEX OY [FI/FI]; Laippatie 1, FIN–00880 Helsinki (FI).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SIPPONEN, Pentti [FI/FI]; Käärmesaarentie 4 A, FIN–02160 Espoo (FI). SUOVANIEMI, Osmo [FI/FI]; Kulopolku 6, FIN–00570 Helsinki (FI). TAMMINEN, Jani [FI/FI]; Ormusmäentie 8 C 45, FIN–00700 Helsinki (FI).

(74) Agent: OY JALO ANT–WUORINEN AB; Iso Roobertinkatu 4–6 A, FIN–00120 Helsinki (FI).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR THE DETERMINATION OF DISACCHARIDASES AND KIT THEREFOR

(57) Abstract

A method for the determination of disaccharidase in a duodenum biopsy sample, which method comprises the steps of: contacting the sample as such with a substrate medium containing a disaccharide; and determining the presence of a desired monosaccharide in the substrate medium by using an assay system for said monosaccharide. The invention is aimed also at a kit for use in carrying out the said method, the kit comprising a substrate medium containing the said disaccharide for contacting with the biopsy sample; and means for the determination of the presence of a desired monosaccharide in the substrate medium after exposure of the substrate medium to the biopsy sample.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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METHOD FOR THE DETERMINATION OF DISACCHARIDASES AND KIT THEREFOR

The present invention relates to a method for the determination of disaccharidases in a biopsy sample from the duodenum, usually in connection with a gastroscopic procedure, of a patient suspected of suffering from a condition of disaccharide intolerance, especially lactose intolerance. The invention also relates to a kit for use in the diagnosis of said intolerance. The present method can easily be carried out as a rapid "bed-side" diagnostic method.

Background of the invention

Disaccharide intolerance is defined as the limited ability of the organism to digest disaccharides, typically milk sugar, i.e. lactose, but also e.g. maltose intolerance is known. The intolerance is due to a decrease in the activity or the concentration of the corresponding disaccharide digesting enzyme, i.e. of lactase (β-galactosidase) in the case of lactose intolerance, which enzyme is produced in the mucous membrane of the small intestine, or duodenum. The enzyme breaks down the disaccharide to simpler sugars that can then be absorbed into the bloodstream.

Normally, when lactose reaches the digestive system, the lactase enzyme hydrolyzes it to D-glucose and D-galactose. The liver then converts the galactose into glucose, which enters the bloodstream and raises the person's blood glucose level. If lactose is incompletely broken down, the blood glucose level does not rise, and a diagnosis of lactose intolerance is confirmed. The resulting condition, although not usually dangerous, may be very distressing. While not all persons deficient in lactase have symptoms, those who do are considered to be lactose intolerant. See generally Buller, H.A. and Grand, R.J., "Lactose Intolerance," Ann. Rev. Med., Vol. 41, pp. 141-148 (1990).

Common symptoms include nausea, cramps, bloating, gas, and diarrhea, which begin about 30 minutes to 2 hours after eating or drinking foods containing lactose. The symptoms are due to the unabsorbed lactose which in the small testine

binds liquid and speeds up the through-put rate to the large intestine, where the bacteria digest the carbohydrates to short chain fatty acids, lactate, carbon dioxide and hydrogen.The severity of the symptoms varies depending on the amount of lactose each individual can tolerate.

Some causes of lactose intolerance are well known. For instance, certain digestive diseases and injuries to the small intestine can reduce the amount of enzymes produced. In rare cases, children are born without the ability to produce lactase. For most people, though, lactase deficiency is a condition that develops naturally over time. After about the age of two years, the body begins to produce less lactase. However, many people may not experience symptoms until they are much older.

Between 30 and 50 million Americans are lactose intolerant. Certain ethnic and racial populations are more widely affected than others. As many as 75 percent of all African-Americans and Native Americans and 90 percent of Asian-Americans are lactose intolerant. In the southern Europe and the Middle East the percentage is about 60, and among arabs as high as 90. The condition is least common among persons of northern European descent, e.g. in Finland 11 % of the population are lactose intolerant, but in the northern Scandinavia, 60 % of the Lapps are lactose intolerant.

Lactose intolerance is conventionally diagnosed using a lactose tolerance test, a hydrogen breath test, a stool acidity test or galactose determination.

The lactose tolerance test is the most common test used for diagnosing lactose intolerance. A blood sample after fasting is taken from the patient for glucose determination, whereafter the patient is given a lactose drink. New blood samples are taken after 20, 40 and 60 minutes. The test shows hypolactasia if clear stomach symptoms develop after 1 to 2 hours after taking the lactose drink and if the increase in the blood glucose level remains below 1.1 mmol/l from the initial value.

The hydrogen breath test measures the amount of hydrogen in the breath. Normally, no hydrogen is detectable in the breath. However, undigested lactose is fermented in the colon by bacteria, a result of which is the formation of many gases, including hydrogen. The hydrogen formed is absorbed from the intestine and carried by the blood stream to the lungs, and exhaled. The patient is given a lactose containing drink, after which the breath is analyzed at regular intervals. Increased hydrogen concentrations in the breath means improper digestion of lactose. The test can be affected by certain foods, medication and smoking.

The stool acidicity test measures lactic acid and other short chain fatty acids produced by colon bacteria by fermenting undigested lactose, which acids can be determined in the stool sample. Galactose can in a simple test be determined in the urine after administration of lactose, the test requiring a semi-quantitative determination method for galactose.

Methods for the determination of disaccharides are previously known, but analysis of the disaccharidase content of a biopsy sample usually requires several steps. First of all, the sample must be homogenized, after which it is incubated with a substrate (lactose, maltose etc.), and then the desired monosaccharide is analysed chemically. The existing methodology is complex and time-consuming. Therefore, there is a need for a single, rapid and specific method of diagnosing disaccharide intolerance, especially lactose intolerance.

The publication EP 72 450 discloses a lactase activity test for infants in conjunction with diagnosing infants for cystic fibrosis (CF), such CF-infants reportedly having increased disaccharidase activities in the meconium. Accordingly, a thin film of a meconium sample is spread on a test device containing lactose, glucose oxidase, a peroxidatively active agent and a chromogen, and if the sample has lactase activity, an easily visible blue colour develops directly beneath the meconium.

Summary of the invention

The present invention provides a quick and easy method for the determination of disaccharidase enzyme in a biopsy sample taken from the duodenum of an individual suspected of being disaccharide intolerant, which method comprises the steps of

- contacting the said biopsy sample as such with a substrate medium containing the said disaccharide; and
- determining the presence of a desired monosaccharide in the substrate medium by using an assay system for said monosaccharide.

It is a further object of this invention to provide a kit for use in carrying out the above mentioned method comprising

- a substrate medium containing the said disaccharide for contacting with a biopsy sample taken from the duodenum of an individual suspected of being disaccharide intolerant; and
- means for the determination of the presence of a desired monosaccharide in the substrate medium after exposure of the substrate medium to the said biopsy sample.

Further areas of applicability of the present invention will be apparent from the detailed description given hereinafter.

Detailed description of the invention

According to the present invention, disaccharide intolerance is diagnosed in an individual by detecting a deficiency or reduced activity of the corresponding disaccharide digesting enzyme, disaccharidase, in a biopsy sample taken from the duodenum of the individual where the corresponding enzyme is normally produced.

Although reference is made specifically to lactose as the disaccharide and lactase as the corresponding disaccharide digesting enzyme, it is clear that the description

equally well applies to methods for diagnosing also other disaccharide intolerance conditions. Such conditions include maltose intolerance, in which case a deficiency of maltase enzyme will be the object of diagnosis, or saccharose intolerance, in which case the enzyme to be diagnosed is saccharidase.

In short, the method comprises detecting the presence of disaccharidase in a biopsy sample taken from the duodenum of an individual suspected of suffering from a condition of disaccharide intolerance, which method comprises a first step of contacting the biopsy sample as such, in intact form, that is in an unprocessed, such as in an unhomogenized and uncomminuted form, with a substrate medium containing the said disaccharide. Any disaccharidase present in the sample digests the disaccharide in the substrate to monosaccharides. In a subsequent step, the presence of a desired monosaccharide so formed in the substrate medium is determined by using an assay system for said monosaccharide.

When the object of diagnosis is lactose intolerance, and the method thus comprises detecting the possible presence or absence of lactase enzyme activity in the biopsy sample, the disaccharide to be used in the substrate medium is lactose. Lactose is digested by any lactase present in the biopsy sample to glucose and galactose, which can be detected in the substrate medium in a known manner.

Maltose, on the other hand, will be digested by the maltase enzyme to two glucose molecules, and saccharose is digested by saccharidase to glucose and fructose.

The method can be carried out in a simple manner, for example by using a substrate medium which in the same solution contains the substrate for the enzyme, that is lactose, if a lactase enzyme deficiency is to be diagnosed, glucose oxidase (or galactose oxidase) enzyme, a peroxidase enzyme and a chromogenic substance. It is also possible to keep one or more of the reagents separate from the other reagents up until the moment of carrying out the test. One such alternative is to keep the chromogenic substance, and/or the glucose or galactose enzyme, in a separate solution, or for example absorbed onto a suitable medium, for example a gel

matrix, or paper, to be contacted with the remaining reagents at the moment of testing. Other modifications of carrying out the test are also possible, and easily construed by a person skilled in the art.

The disaccharidase enzyme in the biopsy sample introduced into the substrate medium will digest the disaccharide in the substrate medium to glucose, galactose and/or fructose, depending on the type of disaccharide. The glucose (or galactose) oxidase enzyme in the same medium, which preferably is buffered to approximately pH 5-7, then oxidizes the glucose or galactose to oxidation products, liberating hydrogen peroxide (H_2O_2). The peroxidase enzyme catalyzes a reaction where the hydrogen peroxide oxidizes the colourless chromogenic substance to form a coloured or otherwise detectable form.

The colour reaction taking place in the substrate is rapid and detectable at room temperature already after a few minutes. The biopsy sample can be a small, e.g. of the order of 1 mm x 1 mm x 1 mm, taken from the duodenum in connection with a gastroscopic procedure. The sample taken is used as such and there is no need to homogenize or otherwise comminute the sample prior to testing. The colour change can be determined either with the bare eye, or can be read with a suitable apparatus e.g. photometrically, fluorometrically or reflectometrically. The method makes it possible to evaluate also the disaccharidase level in the biopsy sample, i.e. to make a semiquantitative analysis, and thus to evaluate the severity of the intolerance condition. The method is easy and rapid to carry out as a 'bedside test' and requires no complicated laboratory equipment.

The concentrations of the various reagents in the substrate medium are not critical and can be adjusted to provide for optimal testing conditions. The reaction can be carried out in a suitable vessel at room temperature, or it can be provided in a suitable kit-form, the kit containing all the reagents needed for carrying out the test in a single ready-to-use package.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

Claims

1. Method for the determination of a disaccharidase enzyme, which is able to digest a disaccharide into monosaccharides, in a biopsy sample taken from the duodenum of an individual to be tested for disaccharide intolerance, which method comprises the steps of
- contacting the said biopsy sample as such with a substrate medium containing the said disaccharide; and
- determining the presence of a desired monosaccharide in the substrate medium by using an assay system for said monosaccharide.

2. The method according to claim 1, wherein the disaccharidase to be determined in the sample is lactase, maltase, or sucrase.

3. The method according to claim 1, wherein the disaccharide is lactose.

4. The method according to claim 3, wherein the monosaccharide to be determined in the substrate medium is glucose.

5. The method according to claim 1, wherein the substrate medium contains disaccharide, glucose and/or galactose oxidase, a peroxidase enzyme and a chromogenic substance.

6. The method according to claim 4, wherein the glucose assay system is a reagent strip, preferably a dip-and-read reagent strip.

7. The method according to claim 1, wherein the assay system for determining the monosaccharide is photometric, fluorometric or reflectometric.

8. Kit for use in carrying out the method according to claim 1, comprising
- a substrate medium containing the said disaccharide for contacting with the biopsy sample; and

- means for determining the presence of a desired monosaccharide in the substrate medium after exposure of the substrate medium to the biopsy sample.

9. The kit according to claim 8, wherein the substrate contains a glucose or galactose enzyme, and a peroxidase enzyme.

10. The kit according to claim 9, wherein the means for the determination of the presence of glucose in the substrate medium comprises a chromogenic substance.

11. The kit according to claim 10, wherein the chromogenic substance is kept separate from the other components of the substrate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00375

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12Q 1/34, C12Q 1/54
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Clinica Chimica Acta, Volume 183, 1989, J.A. Smith et al, "Small bowel biopsy for disaccharidase levels: evidence that endoscopic forceps biopsy can replace the Crosby capsule" page 317 - page 322 --	1-11
X	Clinical Nutrition, Volume 15, 1996, T. H. Iqbal et al, "Small intestinal lactase status, frequency distribution of enzyme activity and milk intake in a multi-ethnic population" page 297 - page 302 --	1-11
A	EP 0072450 A1 (MILES LABORATORIES INC.), 23 February 1983 (23.02.83) --	6,8-11

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
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Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2000	01 -09- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Hampus Rystedt/gh Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00375

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US 5183742 A (KOUICHI OMOTO ET AL), 2 February 1993 (02.02.93), see claims 1-2, and description ---	1-11
A	Annu. Rev. Med., Volume 41, 1990, Hans A. Büller et al, "LACTOSE INTOLERANCE" page 141 - page 148 --- --------	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/FI 00/00375

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE 3273346 D	00/00/00
		JP 1311970 C	11/04/86
		JP 58023797 A	12/02/83
		JP 60036757 B	22/08/85
		US 4524133 A	18/06/85
US 5183742 A	02/02/93	DE 3506365 A,C	29/08/85
		JP 1934225 C	26/05/95
		JP 6053074 B	20/07/94
		JP 60178356 A	12/09/85

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 31863	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/FI 00/00375	International filing date *(day/month/year)* 28 April 2000	(Earliest) Priority Date *(day/month/year)* 30 April 1999
Applicant LOCUS GENEX OY ET AL		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [ ] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the title,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. --
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2000	01 -09- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Hampus Rystedt/gh Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00375

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	Annu. Rev. Med., Volume 41, 1990, Hans A. Büller et al, "LACTOSE INTOLERANCE" page 141 - page 148 --- --------	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

08/05/00

International application No.
PCT/FI 00/00375

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 58023797 A	12/02/83
		JP 60036757 B	22/08/85
		US 4524133 A	18/06/85
US 5183742 A	02/02/93	DE 3506365 A,C	29/08/85
		JP 1934225 C	26/05/95
		JP 6053074 B	20/07/94
		JP 60178356 A	12/09/85